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ENZYME ELECTROPHORESIS OF SOUTH AFRICAN *SCHISTOSOMA MATTHEEI* AND *S. HAEMATOBIUM*

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ABSTRACT

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As a pilot project of a study undertaken to determine the influence of *S. mattheei* × *S. haematobium* hybridization on various South African *S. mattheei* populations by means of biochemical-taxonomic methods, a comparative electrophoretic study of laboratory-maintained *S. mattheei* and *S. haematobium* was performed, using 11 enzymes representing 16 gene loci. Eleven loci were found to be monomorphic, while 5 differed interspecifically. Computation of the results revealed that South African *S. mattheei* and *S. haematobium* are fairly closely related when compared with other *Schistosoma* spp. groups.

INTRODUCTION

As enzymes are transcribed from the DNA contained in the nucleus of an organism, the electrophoretic properties of an enzyme provide a method by which to characterize the gene coding for the enzyme. By studying a range of enzymes in 2 animal species an empirical expression can be obtained as to the relationship between the species.

Kruger, Schutte, Visser & Evans (1986) and Kruger, Hamilton-Attwell & Schutte (1986) observed that populations of the bovine blood fluke *Schistosoma mattheei* which occur sympatric with the human parasite *S. haematobium*, have more phenotypic qualities in common with *S. haematobium* than allopatric populations of *S. mattheei*. They attributed the presence of these qualities to *S. mattheei* × *S. haematobium* hybrids from human origin that have reinfected cattle and introduced *S. haematobium* genes into the genepool of the sympatric *S. mattheei* populations. The most widely used technique in biochemical systematics is electrophoresis (Ferguson, 1980). It was therefore selected as the technique for further study of these populations.

The electrophoretic properties of the enzymes of *S. haematobium* from Africa and the Middle East differ from region to region (Wright & Ross, 1983). Before the ultimate objective, viz. the measurement of the effect of hybridization on the genepool of *S. mattheei*, could be achieved, a baseline comparison of South African *S. mattheei* and *S. haematobium* was essential. In this paper the genotypic and phylogenetic relationships between *S. mattheei* and the local strain of *S. haematobium* were calculated from the degree of correspondence between a selected range of enzymes.

MATERIAL AND METHODS

S. mattheei used in this study was isolated from cattle in the Eastern Transvaal and maintained in *Praomys (Mastomys) coucha*. *S. haematobium* was obtained from man, also from the Eastern Transvaal and was maintained in *Saccostomus campestris*. *Bulinus (Physopsis) globosus* was used as intermediate host snail for both species.

Adult male schistosomes required for electrophoresis was obtained by employing the perfusion technique of Jackson, Dettman & Higgens-Opitz (1982).

Electrophoresis was performed on 220 mm × 140 mm × 2 mm, 12,5 % horizontal starch gels. For each enzyme, the electrophoretic patterns of at least 10 adult males were studied individually. Single worms were macerated in 10 $\mu\ell$ of distilled water and the homogenate absorbed into 5 mm strips of crochet cotton which was inserted into cuts made across the width of the gel. Four enzymes were studied with the homogenate obtained from a single worm.

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Fourteen enzyme systems were studied initially. Eleven of these were selected for further study, since they developed relatively strong and clear electrophoretic patterns. They are: acid phosphatase (ACP), aldolase (ALD), glucose-6-phosphate dehydrogenase (G6PD), glutamate oxalo acetate transaminase (GOT), hexokinase (HK), lactate dehydrogenase (LDH), leucylglycyl glycine aminopeptidase (LGG), malate dehydrogenase (MDH), 1-naphthyl acetate esterase (EST), octanol dehydrogenase (ODH) and phosphoglucomutase (PGM).

The electrode and gel buffer systems used and the references containing the enzyme staining solutions are given in Table 1. Except for LGG, for which a 50 mℓ agar overlay was used, all stains were made up to 100 mℓ.

Electrophoresis was conducted at 16 V per cm gel at 8 °C for approximaely 3 h.

Gels were incubated at 37 °C in the dark until the banding patterns became visible, after which they were photographed.

Nei's (1972) indices of genetic distance ($\bar{D}$) and normalized genetic identity ($\bar{I}$) were calculted using Green's (1979) BASIC programme.

RESULTS

ACP migrated catodically. No variation between the 2 species was observed. A smear extended from the origin to the base of the band (Fig. 1a).

ALD migrated anodically at the same rate in both species and stained as a faint but relatively broad band (Fig. 1b).

EST was represented by 3 monomorphic loci. The first 2 bands (EST-1 & 2) stained alike in both species while the 3rd electromorph (EST-3) stained darker in *S. mattheei*.

G6PD bands were single, dark and well-defined. The enzyme migrated faster in *S. haematobium* than in *S. mattheei* (Fig. 2a).

GOT migrated towards the cathode. The bands were monomorphic, faint, but well-defined (Fig. 1d).

HK was represented by a single band in both species. The *S. haematobium* electromorph migrated slightly faster than the other (Fig. 2b).

LDH was monomorphic. This enzyme stained well, but a smear extended from the origin to the base of the band (Fig. 1e).

TABLE 1 Buffer systems used and the references from which the stain recipes were obtained

Enzyme	Electrophoretic buffer systems		Stain reference
	Electrode buffer	Gel buffer	
Acid phosphatase	0,04 M citric acid pH 7,4	0,002 M citric acid pH6,4	Fletcher *et al.* (1981)
Aldolase	0,3 M boric acid pH 8,2	0,01 M Tris-HC1 pH 8,5	Fletcher *et al.* (1981)
Glucose-6-phosphate dehydrogenase	0,3 M boric acid pH 8,2	0,01 M Tris-HC1. pH 8,5	Fletcher *et al.* (1981)
Glutamate oxalo-acetate transaminase	0,04 M citric acid pH 7	0,002 M citric acid pH 7	Nichols & Ruddle (1973)
Hexokinase	0,3 M boric acid pH 8,2	0,01 M Tris-HC1 pH 8,5	Fletcher *et al.* (1981)
Lactate dehydrogenase	0,04 M citric acid pH 7	0,002 M citric acid pH 7	Fletcher *et al.* (1981)
Leucylglycyl glycine aminopeptidase	0,04 M citric acid pH 7	0,002 M citric acid pH 7	Fletcher *et al.* (1981)
Malate dehydrogenase	0,04 M citric acid pH 7	0,002 M citric acid pH 7	Fletcher *et al.* (1981)
Naphthyl-acetate esterase	0,3 M boric acid pH 8,2	0,01 M Tris-HC1 pH 8,5	Fripp & McSheehy (1969)
Octanol dehydrogenase	0,04 M citric acid pH 7	0,002 M citric acid pH 7	Ayala *et al.* (1973)
Phosphoglucomutase	0,3 M boric acid pH 8,2	0,01 M Tris-HC1 pH 8,5	Fletcher *et al.* (1981)

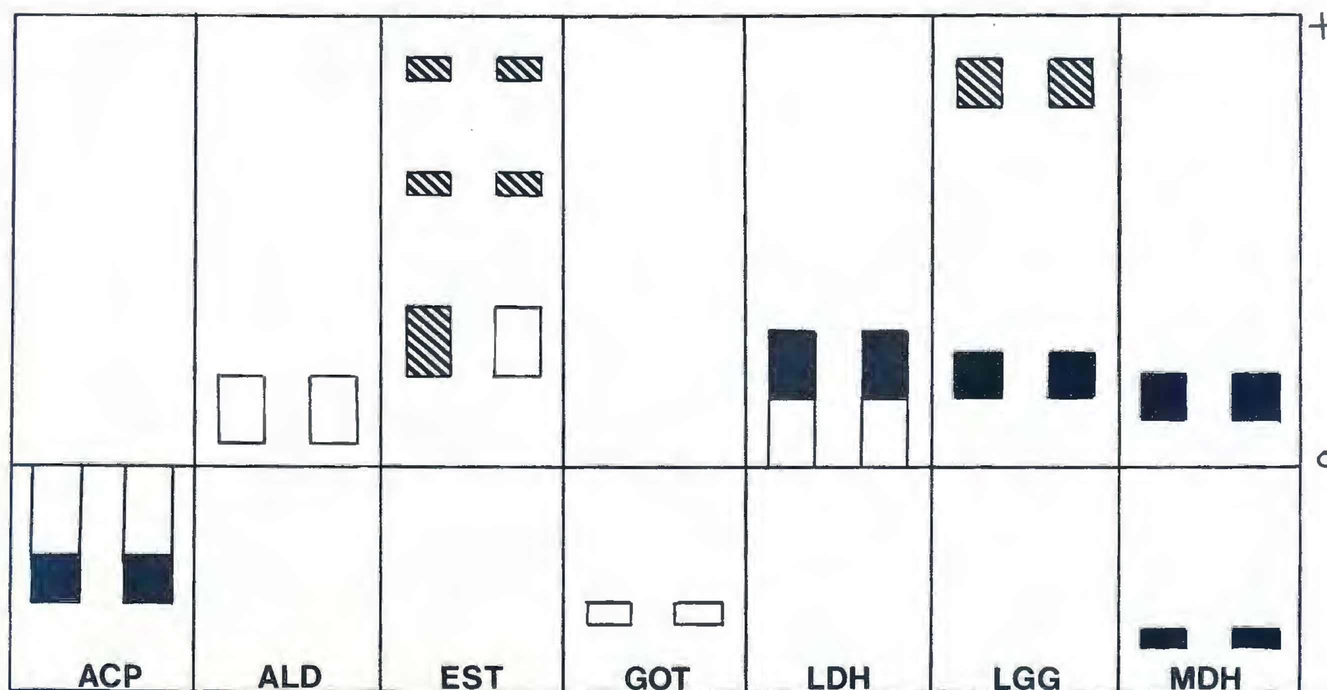

FIG. 1a–g Schematic diagram of the relative mobilities of the 11 monomorphic loci when run under the conditions mentioned in the text. The 3 grades of shading indicate differences in stain intensity. For each locus *S. mattheei's* electromorph is left and *S. haematobium's* right. (See text for enzyme abbreviations)

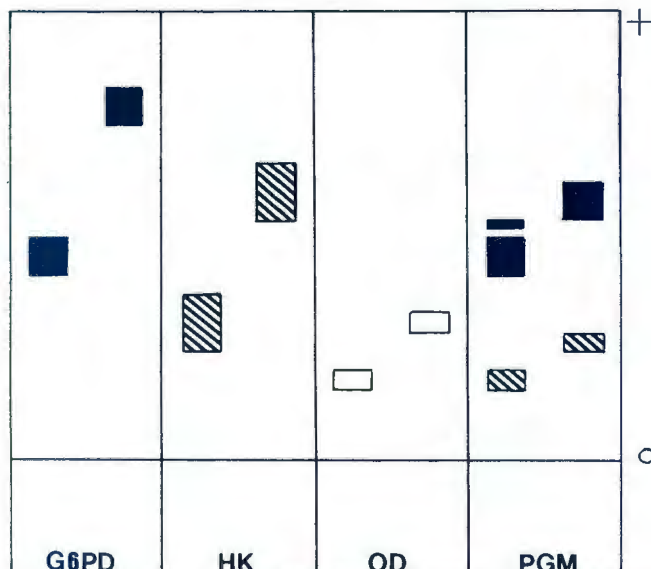

FIG. 2a–d Schematic diagram of the relative mobilities of the 5 dissimilar loci when run under the conditions mentioned in the text. The 3 grades of shading indicate differences in stain intensity. For each locus *S. mattheei's* electromorph is left and *S. haematobium's* right. (See text for enzyme abbreviations)

LGG vizualized as 2 iso-enzymes, LGG-1 and LGG-2 which were identical in both species (Fig. 1f).

MDH is coded for by 2 monomorphic loci. MDH-1 migrated towards the anode and MDH-2 towards the cathode (Fig. 1g).

ODH stained faintly. The band recorded for *S. haematobium* migrated slightly faster than the band recorded for *S. mattheei* (Fig. 2c).

PGM was represented by 2 iso-enzymes, PGM-1 and PGM-2, PGM-1 being double-banded in *S. mattheei* and single in *S. haematobium*. Both the electromorphs migrated faster in *S. haematobium* than in *S. mattheei* (Fig. 2d).

None of the enzymes studied exhibited intraspecific variation.

Thus from an estimated 16 loci observed, 11 were monomorphic and 5 dissimilar. Assimilated in Nei's (1972) indices, an I value of 0,666 and a D value of 0,176 were calculated.

DISCUSSION

Wright & Ross 1980 used G6PD and PGM to study *S. mattheei* × *S. haematobium* hybrids, of human origin, as they found the isoelectric focussing (IEF) patterns of these 2 enzymes to differ between the two species. This observation is confirmed, as in this study they were also found to differ. Ross (1976) compared the IEF patterns of LDH and ACP from South African *S. mattheei* with, amongst other schistosomes, Kenyan *S. haematobium*. He observed a "general similarity of pattern" between the two species for ACP. The LDH patterns of the two species had 4 major fractions in common, but *S. mattheei* exhibited an additional alkaline grouping. The complete range of enzymes reported on in the current study are being further studied by means of IEF.

Fripp (1970) reported that the IEF pattern of EST from *S. mattheei* differed from that of *S. haematobium*. In the current study it was found that although their concentrations differed, the 3 iso-enzymes recorded for the 2 species had similar Rf values on starch gel.

The PGM patterns recorded in the current study correspond with the observations made on the cercariae of the 2 species by Mahon & Shiff (1978), in that the 2 iso-enzymes migrated faster in *S. haematobium* than in *S. mattheei* (Fig. 2d).

According to the Ayala, Tracey, Hedgecock & Richmond (1974) scale, an $\bar{I}$ value of 0,666 indicates that *S. mattheei* and *S. haematobium* are 2 subsepcies of the same species. Using Nei's (1975) formula for the estimation of divergence time ($t = 5 \times 10^6 \bar{D}$, where t is time in years), the estimation may be made that the 2 parasites diverged from a common ancester about 880 000 years ago. This would mean the South African *S. haematobium* and *S. mattheei* are more closely related than, for instance, the 2 human schistosomes, *S. japonicum* and *S. mekongi* (Fletcher, Woodruff, LoVerde & Asch, 1980), which formerly were believed to be synonymous species. A comprehensive electrophoretic study of the phylogenetic relationship between all the African schistosome species having terminal-spined eggs would be interesting.

The schistosome populations used in this study have been maintained in the laboratory for more than 20 years. Fletcher *et al.* (1980) observed no electrophoretic variation (on starch gel) within *S. japonicum*, *S. mekongi* and *S. mansoni* populations kept in rodents for a number of generations, but did record polymorphic variation in F1 *S. mansoni* populations in rodents (Fletcher, LoVerde & Woodruff, 1981). Wright & Ross (1980) also recorded polymorphism within *S. haematobium* populations from various localities in Africa and the Middle East after a single passage through mamsters. It is thus essential that an electrophoretic study on variation amongst South African populations of *S. mattheei* should be done on recently found laboratory populations.

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